ISSN (Print 0974-2115) (Online 2349-8552)
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

A New Validated RP-HPLC Method for the Determination of Emtricitabine and Tenofovir AF in its Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

A Novel Analytical method has been developed for the concurrent evaluation of Emtricitabine and Tenofovir AF by RP-HPLC method. The chromatographic conditions were successfully progressed for the dissociation of Emtricitabine and Tenofovir AF by using Inspire C18column (4.6×250mm) 5µm, moving time was 1ml/min, mobile phase ratio was (30:70v/v) Mixed phosphate buffer (KH_2PO_4 and K_2HPO_4) pH 3 (pH was adjusted with orthophosphoric acid) ACN, detection wave length was selected 273 nm used was WATERS HPLC Auto Sampler, Separation module 2695, UV detector 2497, Empower-software version-2. The assay of Emtricitabine and Tenofovir AF was accomplished with tablets and the % assay was erected to be 99.97 and 100.64 which shows that the method is useful for routine analysis. The linearity of Emtricitabine and Tenofovir AF was found to be linear with a correlation coefficient of 0.999 and 0.999, the approval principle of precision is RSD should be not more than 2.0% and the method show precision 0.4 and 0.8 for Emtricitabine and Tenofovir AF which gives that the method is precise. The approval principle of intermediate precision is RSD should be not more than 2.0% and the technique illustrate ID precision 0.1 and 0.7 for Emtricitabine and Tenofovir AF which proves that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery must exist in the array of 97.0% - 103.0%. The total resurgence was originate to be 99.86% and 99.96% for Emtricitabine and Tenofovir AF. The method development indicates that the accuracy is good inside the limit, which indicates that the technique is capable of showing good accuracy and reproducibility. The reception criterion for Limit of Detection and Limit of Quantification is 3 and 10. The Limit of Detection and Limit of Quantification for Tenofovir AF was originate to be 2.98 and 10.00 and Limit of Detection and Limit of Quantification for Emtricitabine was originate to be 3.00 and 9.98.

KEY WORDS: Emtricitabine, Tenofovir AF, HPLC, ACN.

1. INTRODUCTION

Emtricitabine is the most important nucleoside reverse transcriptase inhibitor (NRTI) used for the treatment of HIV infection in adults. Emtricitabine acts as an analogue of cytidine. By using this drug to inhibiting the reverse transcriptase and the enzyme that copies Human immune deficiency virus RNA into novel viral Deoxyribo nucleic acid.

Tenofovir Alafenamide fumarate (a prodrug of tenofovir), it is available in the form of Viread, it consists of an antiretroviral drugs, called by nucleotide analogue reverse transcriptase inhibitors (nRTIs), which block reverse transcriptase, an enzyme crucial to viral production in HIV-infected people. *In vivo* tenofovir Alfanamide fumarate is currently available to to fixed dosage combination products odefey and descovy. These are the products which are used for the treatment of HIV-1 infection in a person who is fully grown and a person who has reached the age of majority.

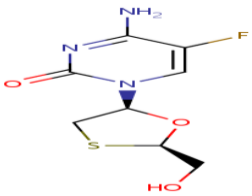

Figure.1. Structure of Emtricitabine

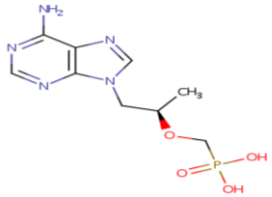

Figure.2. Structure of Tenofovir AF

2. MATERIALS AND METHODS

Instrumentation: The widely used separation technique chromatography was perform on alliance Waters 2695 HPLC system, prepared with an auto sampler, UV detector and Empower 2 software. The detection was carried out at 272 nm with an Inspire (4.6 x 250mm, 5µm) dimensions at ambient temperature.

Chemicals and reagents: Tenofovir AF and Emtricitabine were supplied as gift sample from Mylon laboratories, Hyderabad. KH2PO4&K2HPO4 was analytical grade supplied by FINER chemical LTD, Mumbai, Orthophosphoric acid (Merck), Acetonitrile (Merck, HPLC grade) and Water for HPLC (LICHROSOLV (MERCK).

Preparation of solutions: Preparation of Mixed phophate buffer: 2.95 gm of KH_2PO_4 and 0.54 gm K_2HPO_4 in 1000ml of HPLC water. Sonicate and Ph-3 is adjusted with Orthophopharic acid (0.1%).

Preparation of mobile phase: Assortment a drugs of the solutions like buffer solution 300 ml (30%), 700 ml ACN High Performance Liquid Chromatography (70%) and remove the gas in ultrasonic bath water level for 5 minutes. Vacuum filtration is 0.45 µ.

Diluents Preparation: Use the phase of Mobile as a substance used to dilute.

Preparation of standard stock solution:

Standard Solution Preparation: Take the exact weigh and transmit the 20mg of Emtricitabine 5 mg of Tenofovir AF taken into a 10 milli litres of clean dry titration flask, add the Diluents and silicate to soluble it completely and formulate volume up to the spot with the same solvent (Stock solution).

Hence pipette out 1.5 milli litres of Emtricitabine & Tenofovir AF of the solution is taken into a 10milli litres of Titration flask and mixed up to the mark with Diluents.

Sample Solution Preparation: Take exact weight and move equivalent to 20milli grams of Emtricitabine & 5mg Tenofovir AF equivalent weight of the sample into a 10milli liters of clean and dry Titration flask and mixed the 7milli Liters of Diluents and sonicate to soluble in it totally and make volume up to the mark with the same solvent (Stock solution).

Further pipette out the 1.5ml of Emtricitabine & Tenofovir AF of the stock solution into a 10milli liters Titration flask and mixed up to the mark with Diluents.

Procedure: Inject 20 Micro Liters of the standard, sample into the chromatographic system and measure the areas for Emtricitabine and Tenofovir AF peaks and find out the %Assay with this formulae.

Method development selection of wavelength: Ultra Violet spectrum of 10 µg / ml Emtricitabine and Tenofovir AF in diluents (mobile phase composition) has been recorded by scan in the range of 200nm to 400nm. By using the UV spectrum, wavelength has been selected as 272. Hence wavelength of both drugs show good absorbance.

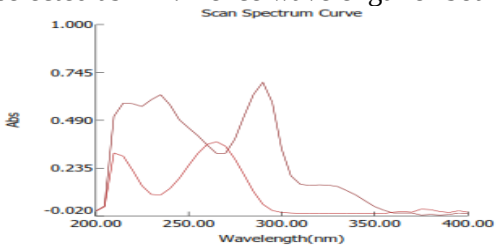

Figure.3. UV Spectra of Tenofovir and Emtricitabinee

Erection of calibration curve: A portion of a different concentrations of standard solution have been prepared and their chromatograms have been recorded at the optimized chromatographic conditions. The mean peak areas at different concentration levels were calculated from the chromatograms. Then the linearity plot was constructed using the mean peak areas at their respective concentrations.

Method validation: The developed method was validated for linearity, accuracy, precision, and limit of detection, limit of quantitation, robustness and Ruggedness system correctness parameters as described in ICH guidelines.

Linearity: From the stock solution, 12.5, 25, 37.5, 50, 62.5 µg/ml solutions for Tenofovir AF and the stock solution, 100, 200, 300, 400, 500 µg/ml solutions for emtricitabine were made and their chromatograms have been recorded. From the recorded chromatograms, their respective mean peak areas have been calculated and the linearity plot has been constructed by using the mean peak areas at different respective concentrations. The coefficient correlation has been found to be 0.999. The linearity data of Tenofovir AF and Emtricitabinee was shown in Table.1 and table.2, the calibration plot.

3. RESULTS AND DISCUSSION

Present investigation has been reported in the notion was intended to develop a new method development and validation for the simultaneous estimation of Emtricitabine and Tenofovir AF by RP-HPLC method. Literature reveals that there are no analytical methods reported for the simultaneous estimation Emtricitabine and Tenofovir AF by RP-HPLC method. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Emtricitabine and Tenofovir AF in pharmaceutical dosage form.

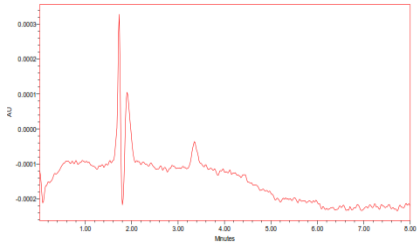

Figure.4. Chromatogram showing blank preparation (mobile phase)

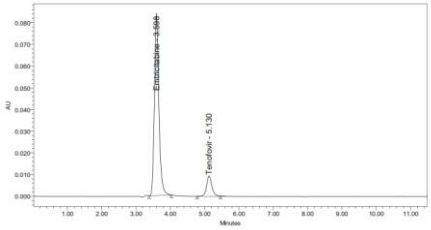

Figure.5. Chromatogram showing assay of STD injection

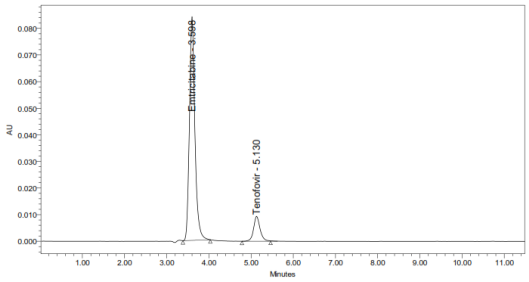

Figure.6. Chromatogram showing standard of sample injection

Table.1. Showing assay results

S. No	Name of compound	Amount taken (mg)	% purity
1	Emtricitabine	200	99.97
2	Tenofovir AF	25	100.64

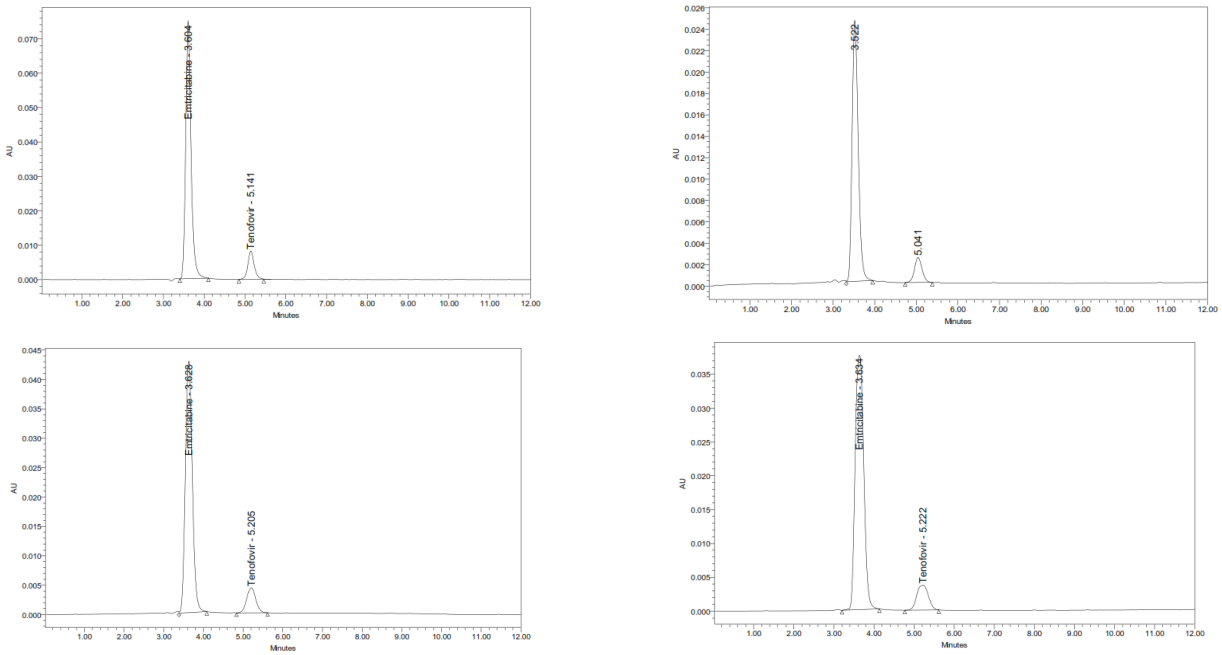

Figure.7. Chromatograms showing linearity overlay

Table.2. Linearity results for Emtricitabinee

S. No	Linearity Level	Concentration (ppm)	Area
1	I	100	244841
2	II	200	525756
3	III	300	856654
4	IV	400	1150925
5	V	500	1435608
Correlation Coefficient			0.999

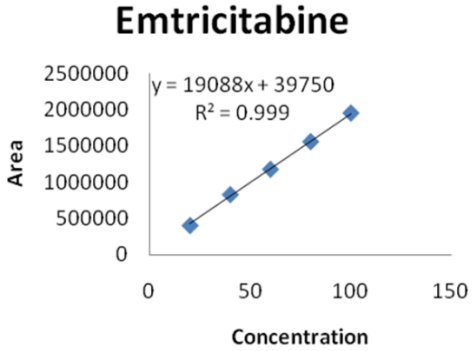

Figure.8. Showing calibration graph for Emtricitabinee

Table.3. Linearity results for Tenofovir

S. No	Linearity Level	Concentration (ppm)	Area
1	I	12.5	29672
2	II	25	68336
3	III	37.5	113345
4	IV	50	159680
5	V	62.5	204473
Correlation Coefficient			0.999

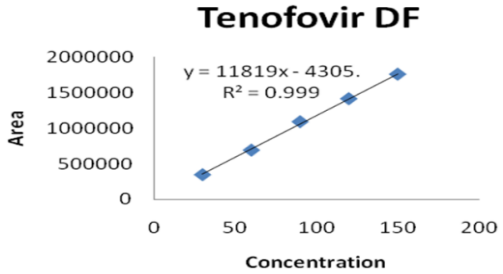

Figure.9. Showing calibration graph for Tenofovir

Accuracy: The accuracy study was performed for 50%, 100% and 150 % for Emtricitabine and Tenofovir. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery.

Table.4. The accuracy results for Emtricitabine

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	427928	10	10.06	99.96	99.86
100%	854989	20	19.97	99.86	
150%	1281399	30	29.93	99.77	

Table.5. The accuracy results for Tenofovir Precision

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	57620	2.5	2.51	100.26	99.96
100%	114986	5	5	100.04	
150%	171648	7.5	7.47	99.56	

The accuracy study was performed for % recovery of Emtricitabine and Tenofovir. The % recovery was found to be 99.86% and 99.96% respectively (NLT 98% and NMT 102%)

Table.6. % RSD results for Emtricitabine and Tenofovir

Injection	Area for Emtricitabine	Area for Tenofovir
Injection-1	852828	111368
Injection-2	852337	112717
Injection-3	858355	112655
Injection-4	852839	113939
Injection-5	858513	113013
Injection-6	857582	112282
Average	855409.0	112662.3
Standard Deviation	3024.5	845.7
%RSD	0.4	0.8

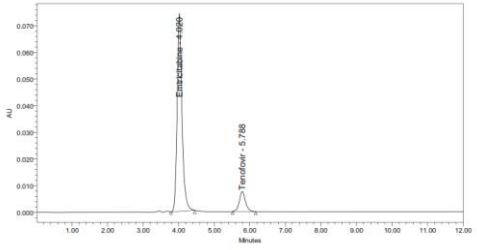

Figure.10. Chromatogram showing less flow

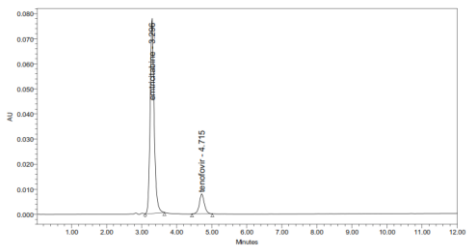

Figure.11. Chromatogram showing more flow

Table.7. Results for intermediate precision of Emtricitabine and Tenofovir

Injection	Area for Emtricitabine	Area for Tenofovir
Injection-1	859453	112535
Injection-2	857162	111224
Injection-3	859458	112915
Injection-4	858377	113391
Injection-5	858482	113108
Injection-6	859771	112959
Average	858783.8	112688.7
Standard Deviation	976.1	769.7
%RSD	0.1	0.7

Table.8. System suitability results for Emtricitabine

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	4361	1.24
2	1.0	3281.91	1.17
3	1.1	4137.68	1.18

Table.9. System suitability results for Tenofovir

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	5749	1.09
2	1.0	4959	1.12
3	1.1	5286	1.04

***** Results for actual flow (1.0ml/min) have been considered from Assay standard.

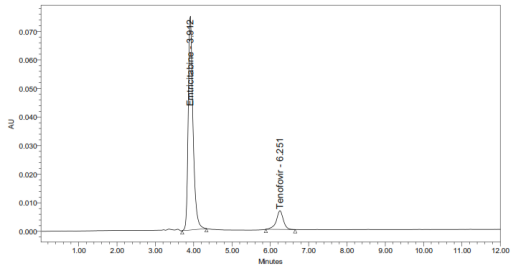

Figure.12. Chromatogram showing less org

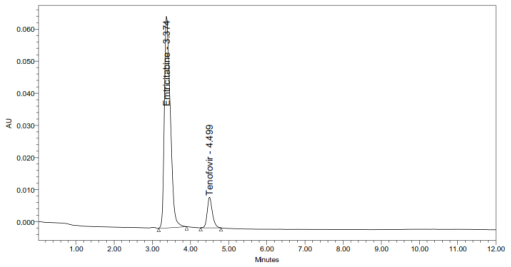

Figure.13. Chromatogram showing more org

Table.10. System suitability results for Emtricitabine

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4962	1.35
2	*Actual	3281.91	1.17
3	10% more	3940	1.44

Table.11. System suitability results for Tenofovir

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6256	0.91
2	*Actual	4959	1.12
3	10% more	5267	1.23

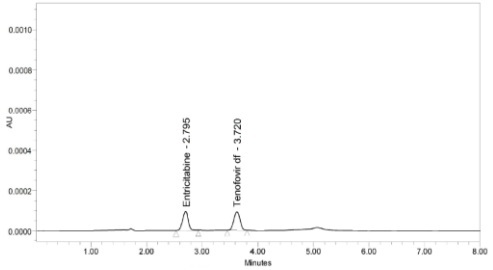

Figure.14. Chromatogram showing LOD

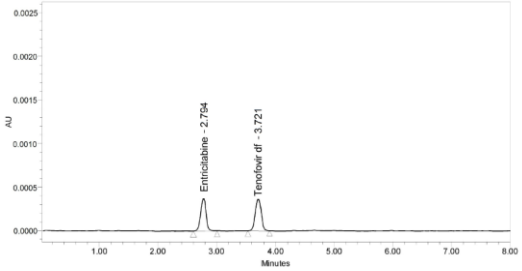

Figure.15. Chromatogram showing LOQ

4. CONCLUSION

The proposed HPLC method was initiate to be simple, precise, accurate and sensitive for the concurrent evaluation of Emtricitabine and Tenofovir AF in pharmaceutical dosage forms. Hence, this method can simply and suitably take up for regular quality control analysis of Emtricitabine and Tenofovir in pure and its pharmaceutical dosage forms.

5. ACKNOWLEDGEMENT

Authors are thankful to the Pharma Train Lab, Kukatpally, for providing instrumental and analytical support.

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